INFLUENCE OF EXPERIMENTAL ACIDOSIS ON GLUCOSE TOLERANCE IN NORMAL & ADRENALECTOMIZED DOGS & OBSERVATIONS ON THE DECREASED ABILITY OF THE LATTER TO RESTORE SERUM BICARBONATE

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Title

INFLUENCE OF EXPERIMENTAL ACIDOSIS ON GLUCOSE TOLERANCE IN NORMAL AND ADRENALECTOMIZED DOGS AND OBSERVATIONS ON THE DECREASED ABILITY OF THE LATTER TO RESTORE SERUM BICARBONATE.

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Introduction

In 1913 Elias observed an elevation of blood sugar following intravenous administration of hydrochloric acid (1). This observation was confirmed in 1927 by Field and Newburgh (2). Much thought has been given to the mechanism of the hyperglycemia accompanying experimental acidosis. Haldane, for example, believed the hyperglycemic effect of acidosis to be due to an inability of the organism to store glucose rather than to a disturbance of glucose oxidation. He based his conclusion on the observation of decreased glucose tolerance without ketonuria or change in the respiratory quotient that followed the ingestion by man of ammonium chloride (3,4).

Facilitation of glucose storage is one of the main functions attributed to insulin. It might be hypothesized on the basis of Haldane's results that experimental acidosis interfered with the function of insulin. In line with such a hypothesis are observations by Guest and Rapoport who studied the influence of acidosis on organic phosphate esters present in red blood cells (5). It is an established fact that the severe acidosis present in precomatose and comatose diabetics is accompanied by a significantly decreased effectiveness of insulin. No valid explanation for the decreased insulin sensitivity is available as yet, as can be seen from the following quotation. "It is generally recognized that patients with severe diabetic acidosis are peculiarly refractory to the action of insulin... The cause of this resistance has not been ascertained...." (6).

It was questioned whether such an effect of acids on insulin sensitivity might be related to a stimulation of the pituitary-adrenal system. If acidosis were accompanied by an increased output of carbonhydrate-active steroids from the adrenals, decreased

insulin sensitivity would have to follow (7). To test this hypothesis, the effect of experimental acidosis on the carbohydrate tolerance of normal dogs was compared with the effect on adrenalectomized dogs. As will be brought out later, additional observations were made on the resistance to acidosis of normal untreated adrenalectomized dogs, as well as adrenalectomized dogs given adrenal extracts and/or desoxycorticosterone.

Materials and Methods

The experiments were performed on trained, unanesthetized, mongrel dogs. Adrenalectomy was carried out as a two stage procedure, observing aseptic technique; dorsolumbar incisions were utilized for exposure. The dogs were anesthetized by intravenous injection of nembutal. The right adrenal was removed one week previous to the left adrenal. The second procedure lasted approximately fifteen minutes from incision to closure. Following the removal of the second adrenal the dogs were continued on the stock kennel food; however, drinking water was changed from tap water to one per cent saline. Full substitution therapy was carried out routinely for three to four days postoperatively with 1cc. "Lipo-Adrenal Cortex" (Upjohn) and 1cc. of "Adrenal Cortex Extract" (Upjohn) per day. After this period the extracts were totally or partially withdrawn or replaced by desoxycorticosterone, as indicated in the description of the particular experiments. The desoxycorticosterone used was Organon's "DOCA Acetate", a solution of desoxycorticosterone acetate in sesame oil.

The HCl solutions used for the production of acidosis were dilutions of stock 1 N HCl. The glucose solution used for intravenous tolerance tests was obtained from sterile ampules of 50% Dextrose in water, manufactured by the "Philadelphia Ampoule Laboratories". The blood for chemical analysis was withdrawn with a syringe containing one drop of heparin and immediately placed under oil. The blood samples were all venous. ~~The blood samples were all venous.~~ Serum bicarbonate (1)* determinations were carried out by the method of Van Slyke and Neill (8), blood sugar by the method of Hagedorn and Jensen (9). Serum electrolyte determinations were performed on a flame photometer by the

by the Department of Internal Medicine. (2)*

Previous to an experiment the dogs were fasted for from twelve to eighteen hours. The hydrochloric acid infusions lasted ten to twenty minutes depending on the volume of fluid to be introduced. The glucose tolerance tests were performed by injection of one gram of glucose per kilo, as 50% glucose in water, injected over a period of fifteen seconds. At the end of the intravenous infusion of acid a blood sample was taken, the needle left in place, and the glucose injected immediately thereafter. The following blood samples were taken at the indicated intervals.

Footnote (1)* The expression of serum CO_2 content as bicarbonate concentration in the absence of serum pH determinations is of course an approximation. However, it is felt that this approximation is valid for the present discussion.

Footnote (2)* The author is indebted to Miss Pauline Hald for the performance of these determinations.

Results

Glucose Tolerance Before and After Production of Acidosis in Normal Dogs -

Table I contains the data obtained from four normal dogs. It is evident that the blood sugar values obtained in glucose tolerance experiments are significantly higher during acidosis than at normal bicarbonate values. In confirmation of previous observations by Elias (1), there is a slight but significant rise in blood sugar during the infusion of the acid; i.e., before the injection of glucose. Figure 1, containing the data obtained from dog #3, illustrates this relationship.

Glucose Tolerance During Acidosis in Adrenalectomized Dogs -

As can be seen from experiments b and c in Table II, there is no significant difference between the response of normal dogs and a bilaterally adrenalectomized dog maintained with DOCA. The data from dog #2 (Table I), and Figures 2 and 3 suggest that the degree of decreased tolerance is related to the degree of acidosis, since the level of bicarbonate is inversely related to the height of the blood sugar curve.

The Response of Adrenalectomized Dogs to Acidosis -

It was recognized early in the course of this study that an amount of acid that will produce a mild acidosis in normal dogs, in adrenalectomized dogs causes very severe acidosis leading easily to fatality. However, on further investigation, it has been found that the lowest bicarbonate values reached immediately after termination of the infusion of acid does not seem to differ significantly between normal and adrenalectomized dogs. Whereas the bicarbonate values of the former start to increase promptly and continue to rise until they approach the pre-experiment level,

the values of the latter, in the post-infusion period, either remain at their lowest level (Table III, Exp. a) or even continue to decline (Table IV).

The similarity of the bicarbonate values at the end of the infusion would indicate that the buffer capacity of the blood has not decreased markedly in the adrenalectomized dog kept only on saline. The difference then must lie in some other factors responsible for the restoration of acid-base equilibrium.

The question arose as to whether substitution therapy of the cortex is able to prevent this inability to restore the serum bicarbonate. Indeed, an adrenalectomized animal kept on full substitution therapy behaved like normal animals; i.e., the bicarbonate values approach normal in the post-infusion period (Table II, Exp. a).

It was highly interesting to observe that the same steady return to normal bicarbonate values also occurred in adrenalectomized dogs kept exclusively on large amounts of DOCA (Table II, Exp. b and c; Table III, Exp. c, d and e). When in further studies on the same adrenalectomized dog the daily maintenance amount of DOCA was decreased from 5.0 mg. to 0.5 mg., it was observed that with this decreasing amount of DOCA the return of bicarbonate values in the post-infusion period proceeded at a slower rate (Figure 4). If DOCA was completely omitted the post-infusion values remained at their low levels, or as mentioned above, they decreased still further.

In line with the above statement concerning the immediate post-infusion bicarbonate values, the few data on sodium, chloride, and potassium concentrations in plasma obtained at the start of the previously described experiments indicate no significant differences between normal and adrenalectomized animals.

Table I

Glucose Tolerance Before and After Production of Acidosis in Normal Dogs

Dog	Acid	Determination	Normal	End of acid infusion	5 Min.*1	30 Min.	120 Min.
#2	4mM/Kg. N/5 HCl	B.S.*2	95	-	257	98	60
		HCO_3*3	52.4	-	37.9	44.0	45.8
	6.5mM/Kg. N/4 HCl	B.S.	82	124	287	192	102
		HCO_3	40.8	16.3	8.9	18.9	20.3
#3	None	B.S.	72	-	192	102	70
	5¼ mM/Kg. N/4 HCl	B.S.	61	82	335	180	118
		HCO_3	44.2	7.6	14.7	27.4	33.2
#4	None	B.S.	129	-	226	102	81
	6mM/Kg. N/4 HCl	B.S.	88	107	330	183	72
		HCO_3	54.7	10.5	22.2	28.9	34.7
#6	None	B.S.	95	-	268	95	58
	6¼mM/Kg. N/4 HCl	B.S.	79	107	309	201	107
		HCO_3	45.4	16.7	20.2	23.6	23.1

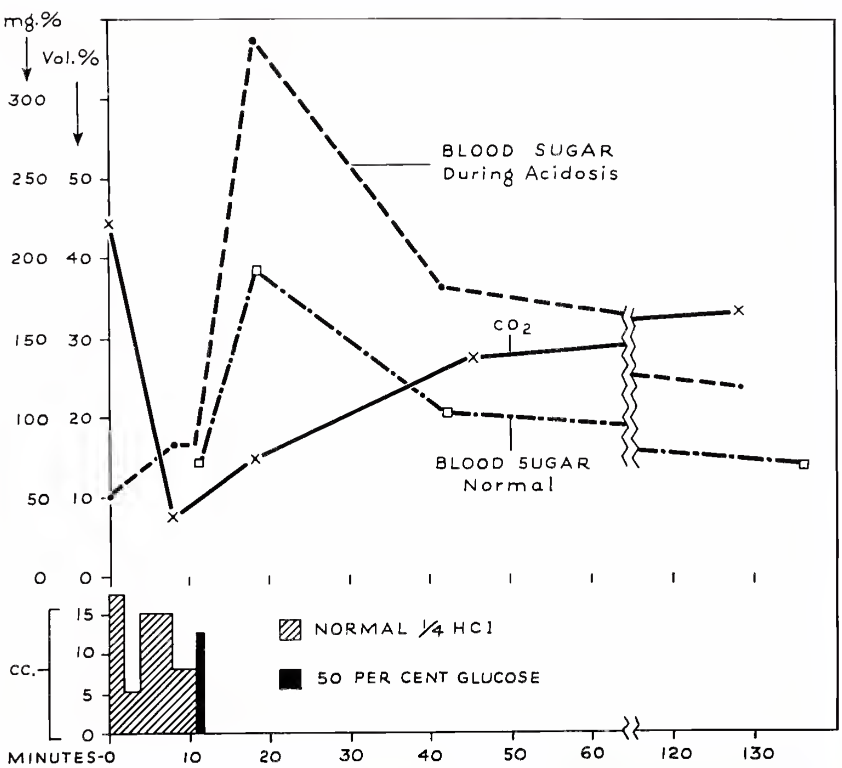
*1 Time intervals measured from end of glucose injection.

*2 Blood Sugar in mg.%.

*3 Serum bicarbonate in Vols.%.

Figure 1.

Data from Dog #3 (Table I)



Glucose tolerance curves in a normal dog before and during acidosis.

Table II

Dog #7 Adrenalectomized.

Daily Maintenance Therapy	Acid	Determination	Normal	End of Acid Infusion	5 Min.	30 Min.	60 Min.	120 Min.
a) 5.0 mg.DOCA	$2\frac{1}{2}$mM/Kg.	B.S.	58	50	260	185	100	65
*1cc.L *1cc.A	N/6 HCl							
(5 days)		HCO_3	50.8	39.9	-	38.9	39.7	46.4
b) 5.0 mg.DOCA	$2\frac{1}{2}$mM/Kg.	B.S.	73	77	231	79	63	61
(2 days)	N/6 HCl	HCO_3	58.3	46.8	47.0	51.6	50.2	51.2
c) 5.0 mg. DOCA	4mM/Kg.	B.S.	84	70	311	183	84	49
(3 days)	N/6 HCl	HCO_3	66.4	40.5	38.2	34.7	38.0	43.0

*1cc.L Lipo-Adrenal Cortex (Upjohn)

*1cc.A Adrenal Cortex Extract (Upjohn)

Table III

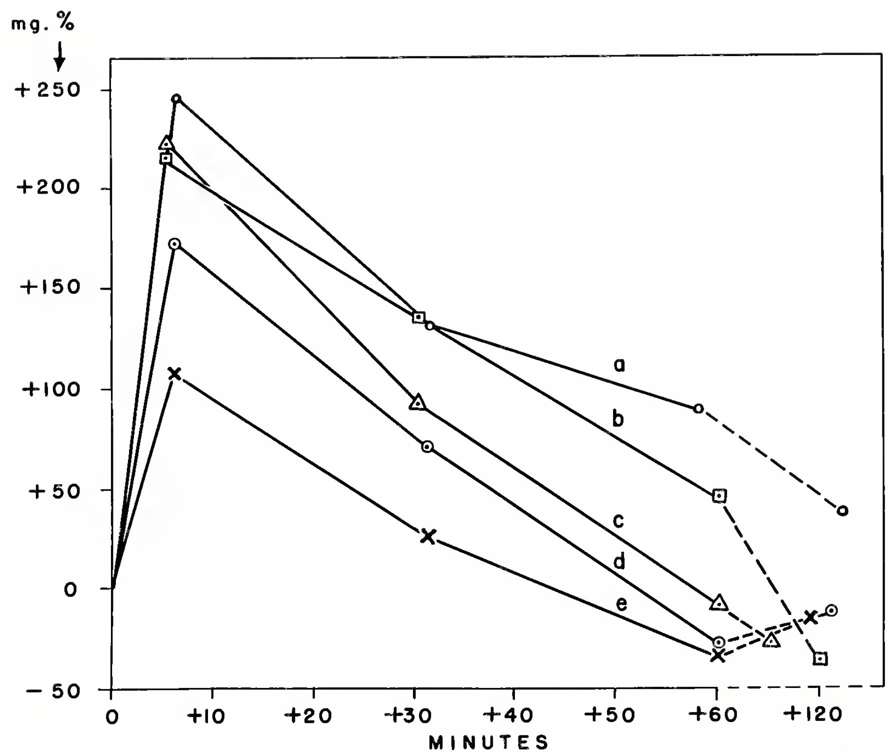
Dog #9 Adrenalectomized

Daily Maintenance Therapy	Acid	Determination	Normal	End of Acid Infusion	5 Min.	30 Min.	60 Min.	120 Min.
a) None	$7\frac{1}{2}$mM/Kg.	B.S.	68	77	322	209	165	116
(7 days)	N/4 HCl	HCO_3	58.2	29.9	25.0	19.6	25.7	27.1
b) 0.5 mg. DOCA	$7\frac{1}{2}$mM/Kg.	B.S.	81	95	311	230	141	59
(6 days)	N/4 HCl	HCO_3	58.8	26.1	26.5	27.9	32.8	35.9
c) 5.0 mg. DOCA	$7\frac{1}{2}$mM/Kg.	B.S.	72	72	293	165	63	45
(2 days)	N/4 HCl	HCO_3	60.6	32.5	36.3	35.9	41.7	44.3
d) 5.0 mg. DOCA	$5\frac{1}{2}$mM/Kg.	B.S.	82	77	251	148	50	66
(4 days)	N/4 HCl	HCO_3	66.3	41.8	41.6	40.6	45.5	-
e) 5.0 mg. DOCA	4mM/Kg.	B.S.	79	77	194	104	45	63
(4 days)	N/4 HCl	HCO_3	74.4	48.7	48.4	48.7	54.9	56.8

The experiments were performed in the order e, d, c, b, a. The dog expired approximately 10 hours after the completion of experiment a.

Figure 2.

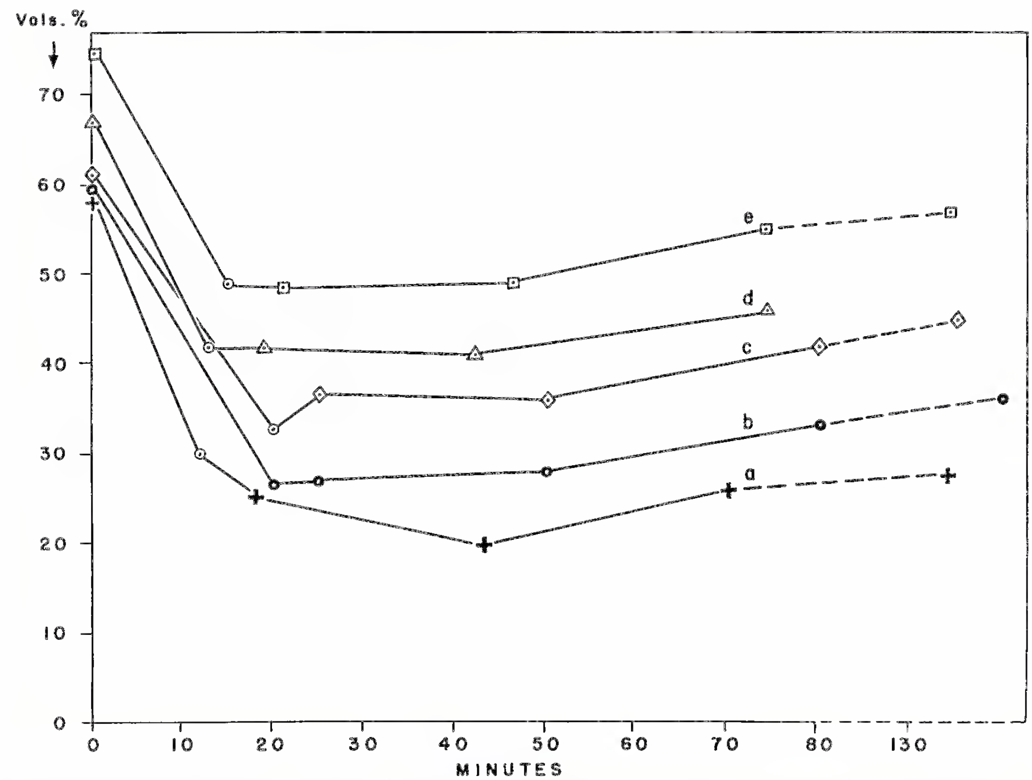
Data from Table III



Glucose tolerance curves in an adrenalectomized dog during different degrees of acidosis. Increasing degrees of acidosis in the following order: e, d, c, b, a. Compare with Figure 3 containing the values of bicarbonate concentrations for these different curves. The blood sugar level before the injection of glucose is taken as zero.

Figure 3.

Data from Table III

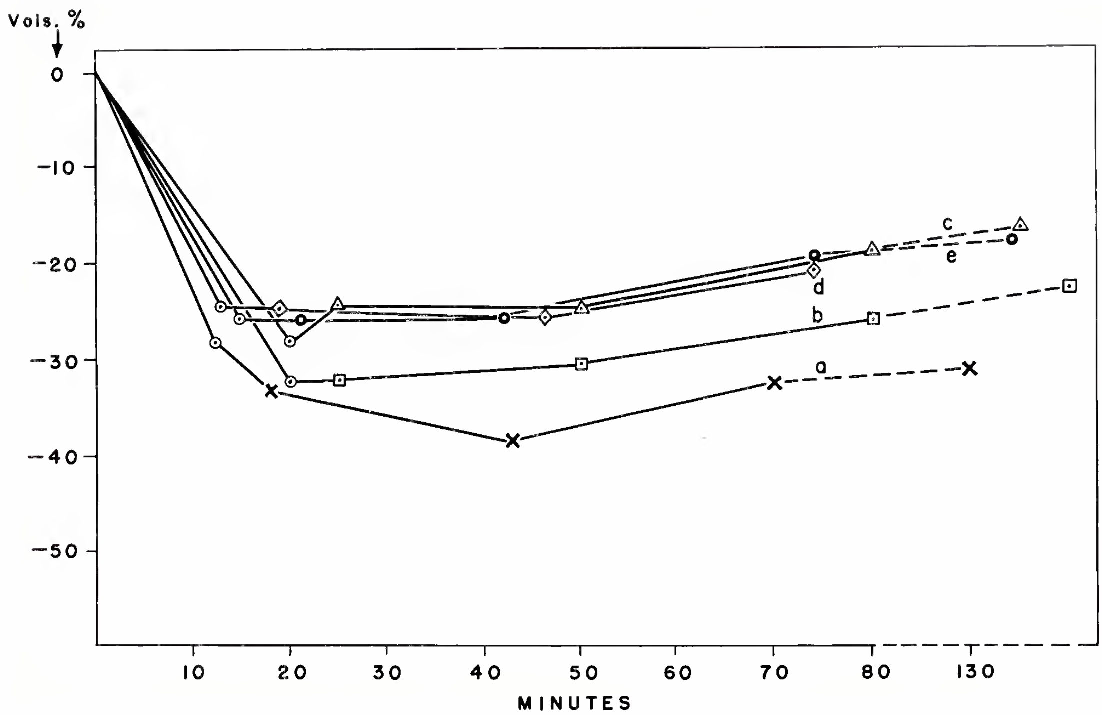


Bicarbonate concentrations following acid infusion into an adrenalectomized dog. The corresponding glucose tolerance curves are contained in Figure 2.

⊙- This symbol indicates the completion of the acid infusion.

<u>Figure 4.</u>

Data from Table III



Bicarbonate concentrations following acid infusion into an adrenalectomized dog. The values represent the fall of bicarbonate from the pre-injection level, indicated as zero.

c,d,e: The dog was treated with 5.0 mg. DOCA daily.

Acid amounts infused - c - $7\frac{1}{2}$ mM/Kg.

d - 5 mM/Kg.

e - $2\frac{1}{2}$ mM/Kg.

b: The dog was treated with 0.5 mg. DOCA daily.

a: The dog was treated with no DOCA.

Acid amounts infused - b - $7\frac{1}{2}$ mM/Kg.

a - $7\frac{1}{2}$ mM/Kg.

Table IV

Dog #10 adrenalectomized

Daily Maintenance Therapy	Acid	Determination	Normal	End of Acid Infusion	5 Min.	30 Min.	60 Min.	90 Min.*
None (4 days)	$7\frac{1}{2}$mM/Kg. N/4 HCl	B.S.	46	59	237	135	104	126
		HCO_3	52.8	22.4	24.6	23.5	21.3	9.3

*Animal died at this point. Blood sample obtained from right heart while heart was still beating.

Discussion

Acidosis - Carbonhydrate Metabolism -

If experimental acidosis were to lead to an increased output of carbohydrate-active steroids from the adrenal cortex, and if this output should be responsible for the decrease of carbonhydrate tolerance, differences of glucose tolerance between adrenalectomized and non-adrenalectomized animals during the acidosis should be demonstrable. Since this was not the case, it can be assumed that this stimulation was lacking. In line with such an assumption are unpublished observations by Fry who found that administration of ammonium chloride to rats did not lead to a decrease in adrenal ascorbic acid (10).

To what degree a long-standing acidosis contributes to the decrease in insulin sensitivity in acidotic diabetics can not be concluded from the above experiments. The observed increased output of carbohydrate-active steroids in diabetic acidosis (11) might contribute significantly to the decrease in insulin sensitivity so characteristic for this acidosis, even if an influence of the adrenal on glucose tolerance could not be demonstrated in the acute experiments reported in this paper.

In any consideration of the possible mechanism of the action of acidosis on carbohydrate metabolism thought should not be confined solely to interference with the action of insulin. It should not be overlooked that acidosis is directly related to many changes in the organism which are in themselves capable of affecting carbohydrate metabolism. In 1924 Haldane described the occurrence of negative balances of phosphate, potassium, and calcium during ammonium chloride acidosis in man (4,12). Many results have been obtained since the publication of these experiments which intimately relate these inorganic ions to carbohy-

drate metabolism.

From observations of the change of serum inorganic phosphate (SIP) concentration after administration of glucose (13) or insulin (14,15), it has been concluded that SIP is in some way connected with the transfer of glucose across cell membranes (16,17). Whether movements of glucose can be initiated by changes in the SIP concentration is still in dispute. Some investigators have observed decreased glucosuria following phosphate administration to diabetics (18,19). In the rat, however, administration of phosphate with glucose does not significantly alter the glucose tolerance (20).

In contract to the uncertainty concerning the effect of phosphate or carbohydrate metabolism, definite information exists with regard to the role played by potassium. It has only recently been demonstrated that a cellular deficiency of potassium will decrease the glucose tolerance of rats (21). It is questionable whether a cellular deficiency of potassium of sufficient degree to produce such an effect was produced by the acidosis herein described.

The studies of Underhill in 1914 were the first to suggest a relationship between serum calcium concentration and blood sugar. This author demonstrated that a low blood sugar following thyreoparathyroidectomy could be restored to normal by injection of calcium (22,23). This low blood sugar was not observed by other investigators (13). It was later shown, however, that the glucose tolerance of a parathyroidectomized dog was directly related to its serum calcium concentration at the start of the test (13). These authors did not take into consideration the possible variations of phosphate under these experimental condi-

tions, thus necessitating further work to establish the implied relationship.

There is much evidence, therefore, to associate the movements of phosphate, calcium, and potassium known to occur during acidosis with the observed decreased glucose tolerance. However, complete balance studies measuring both intra and extracellular electrolytes will be required before any definite statement can be made as to the extent to which they are involved in the demonstrated effect of acidosis on carbohydrate metabolism.

Acidosis - Adrenal Glands -

In the course of studies on the relationship of acidosis to glucose tolerance, it was observed that the ability of the adrenalectomized dog to compensate for an experimental acidosis is greatly diminished as compared with the normal dog. It was further observed that DOCA is capable of preventing this deficiency. Pitts has observed a similar phenomenon in rats in which he produced chronic acidosis with daily injections of ammonium chloride (24).

The decreased ability of the adrenalectomized animal to conserve sodium seems to offer an appropriate explanation for this observation. By the uninhibited urinary excretion of sodium following the administration of chloride, the amount of sodium available in the extracellular fluid for formation of bicarbonate has to diminish progressively. Pitts, however, offers an alternate explanation. This author feels that the defect in the adrenalectomized animals may be found in the decreased ability of their kidneys to produce ammonia, thereby preventing the body from conserving sodium. Jiminez-Diaz has also concluded that the adrenal steroids have a primary effect upon renal ammonia production (25). His conclusions are based on results of in vitro deamination

experiments using dl-alanine as substrate. Since it is now known that glutamine is the major source of urinary ammonia (26), these experiments do not seem to be conclusive. The diminished ammonia production observed by Pitts in his ammonium chloride injected rats and by Loeb, et al (27), and Jiminez-Diaz (25) in patients with Addison's disease can be adequately explained by the established primary inability of the renal tubules to reabsorb sodium. The question as to whether there is an additional direct effect of the steroids on renal ammonia production remains unanswered. This problem is the subject of further study.

Summary

1) Experimental acidosis will decrease the glucose tolerance of dogs independent of the presence or absence of the adrenal glands.

2) The height of the blood sugar curve varies inversely with the level of serum bicarbonate.

3) The adrenalectomized dog cannot restore his serum bicarbonate concentration following an infusion of hydrochloric acid as does the normal dog.

4) DOCA administration restores the ability of the adrenalectomized dog to compensate for the experimental acidosis in the same way as the normal dog.

5) Possible mechanisms involved in the effect of acidosis on carbohydrate metabolism, and in the inability of the adrenalectomized dog to compensate for experimental acidosis have been discussed.

Bibliography

1. Elias, H.,-Über die Rolle der Saüre im Kohlenhydratstoffweckul über Saüre-diabetes.

Biochem. Z. 48, 120, 1912-1913

2. Field, H., Jr. and Newburgh, L.H., Insulin utilization in acidosis.

J. Clin. Invest. 4, 447, 1927

3. Haldane, J.B.S., Wigglesworth, V.B., and Woodrow, C.E., The effect of reaction changes on human carbohydrate and oxygen metabolism.

Proc. Royal Soc. of London 96B, 15, 1924

4. Haldane, J.B.S., Experimental and therapeutic alterations of human tissue alkalinity.

Lancet 1, 537, 1924

5. Guest, G.M., and Rapoport, S., Role of acid-soluble phosphorus compounds in red blood cells in experimental rickets, renal insufficiency, pyloric obstruction, gastroenteritis, ammonium chloride acidosis and diabetic acidosis.

Am. J. Dis. Child 58, 1072, 1939

6. Peters, J.P. and Van Slyke, D.D., Quantitative clinical chemistry. 2nd. Ed. Vol. 1, Interpretations, Baltimore, 1946. Williams & Wilkins Co. Pg. 323.

7. Long, C.N.H., Kutzin, B., and Fry, E.G., The adrenal cortex and carbohydrate metabolism endocrinology 26, 309, 1940

8.a) Van Slyke, D.D., and Neill, J.M., The determination of gases in blood and other solutions by vacuum extraction and manometric measurement.

J.B.C. 61, 523, 1924

b) Van Slyke, D.D., Note on a portable form of the manometric

apparatus, and on certain points in the technique of its use.

J.B.C. 73, 121, 1927

9.a)Hagedorn, H.C., and Jensen, B.H., Zur Mikrobestimmung des Blutzuckers mittels Ferricyanid.

Biochem. Z. 135, 46, 1923

b)Hagedorn, H.C., and Jensen, B.H., Die Ferricyanidmethode zur Blutzuckerbestimmung. II.

Biochem. Z. 137, 92, 1923

10. Fry, E.G., Personal communication.

11. McArthur, J.W., Sprague, R.G., and Mason, H.L., The urinary excretion of corticosteroids in diabetic acidosis.

J. Clin. Endocrinology 10, 307, 1950

12. Haldane, J.B.S., Wigglesworth, V.B., and Woodrow, C.E., The effect of reaction changes on human inorganic metabolism.

Proc. Royal Soc. of London 96B, 1, 1924

13. Salvasen, H.A., The physiology of the parathyroids.

Acta Med. Scandinavia 1926 Supplement VI.

14. Wigglesworth, V.B., Woodrow, C.E., Smith, W., and Winter, L.B., On the effect of insulin on blood phosphate.

Journ. of Physiology 57, 447, 1922-1923

15. Levine, R., Loube, S.D., and Weisberg, H.F., Nature of the action of insulin on the level of serum inorganic phosphate.

American J. of Physiology 159, 107, 1949

16. Peters, J.P., and Van Slyke, D.D., Quantitative clinical chemistry. Vol 1., Interpretations Baltimore, 1932, Williams and Wilkins Co.

17. Peters, J.P., Differentiation and Specialization of biologic media; Research in medical science, Green, D.E., and Knox, W.E., (Editors), New York, 1950, The Macmillan Co., Pgs. 205-236.

18. Adlersberg, D., Aceton urie und Acidose

Biochem. Z. 143, 527, 1923

19. Friedlander, K., and Rosenthal, W.G., Über den Einfluss des Phosphorsaüreions auf den Blut-und Harnzucker des normalen und des diabetisehen organismus.

Arch. Exp. Path. Pharm. 112, 65, 1926

20. Morita, Y., and Orten, J.M., Effect of phosphate upon the utilization of glucose by the alloxan diabetic rat.

American J. of Physiology 162, 416, 1950

21. Gardner, L.I.; Talbot, N.B.; Cook, C.D.; Berman, H.; Concepcion, U.R.; The effect of potassium deficiency on carbohydrate metabolism.

J. Lab. Cl. Med. 35, 592, 1950

22. Underhill, F.P., and Blatherwick, N.R., Studies in carbohydrate metabolism; VI. The influence of thyreoparathy roidectomy upon the sugar content of the blood and the glycogen content of the liver.

J.B.C. 18, 87, 1914

23. Underhill, F.P., and Blatherwick, N.R., Studies in carbohydrate metabolism; VII. The influence of subcutaneous injection of dextrose and of calcium lactate upon the blood sugar content and upon tetany after thyreoparathyroidectomy.

J.B.C. 19, 119, 1914

24. Pitts, R.F., Acid-Base regulation by the kidney.

American J. Med. 9, 356, 1950

25. Jiminez-Diaz, C., Death in Addison's Disease.

Lancet 2, 1135, 1936

26. Van Slyke, D.D., Phillips, R.A., Hamilton, P.B., Archibald, R. M., Futcher, P.H., Hiller, A., Glutamine as a source of urinary ammonia.

J.B.C. 150, 481, 1943

27. Loeb, R.F., Atchley, D.W., and Stahl, J., The role of sodium in adrenal insufficiency.
J.A.M.A., 104, 2149, 1935